Hair glucocorticoids as biomarker for endogenous Cushing's syndrome: validation in two independent cohorts

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Abstract: Background/Aims: The current diagnostic workup of Cushing's syndrome (CS) requires various tests which only capture short-term cortisol exposure, whereas patients with endogenous CS generally have elevated long-term cortisol levels. Scalp hair assessment has emerged as a convenient test in capturing glucocorticoid concentrations over long periods of time. The aim of this multicenter, multinational, prospective, case-control study was to evaluate the diagnostic efficacy of scalp hair glucocorticoids in screening of endogenous CS. Methods: We assessed the diagnostic performances of hair cortisol (HairF), hair cortisone (HairE), and sum of both (sumHairF+E), as measured by state-of-the-art LC-MS/MS technique, in untreated patients with confirmed endogenous CS (n=89), and community controls (n=295) from the population-based Lifelines cohort study. Results: Both glucocorticoids were significantly elevated in CS patients when compared to controls. High diagnostic efficacy was found for HairF (area under the curve (AUC), 0.87 [95% CI, 0.83 to 0.92]), HairE (0.93 [0.89 to 0.96]) and sumHairF+E (0.92 [0.88 to 0.96]; all P<.001). Participants were accurately classified at optimal cut-off threshold in 86% of cases (81% sensitivity, 88% specificity, 94% negative predictive value (NPV)) for HairF, in 90% of cases (87% sensitivity, 90% specificity, 96% NPV) for HairE, and 87% of cases (86% sensitivity, 88% specificity, 95% NPV) for the sum. HairE was shown to be most accurate in differentiating CS patients from controls. Conclusion: Scalp hair glucocorticoids, especially hair cortisone, can be seen as a promising biomarker in screening of CS. Its convenience in collection and workup additionally makes this feasible for first-line screening

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Hair Glucocorticoids as a Biomarker for Endogenous Cushing's Syndrome: Validation in Two Independent Cohorts

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Keywords
Hair analysis · Glucocorticoids · Diagnostics · Cushing's syndrome · Cortisol · Cortisone

Abstract
Background/Aims: The current diagnostic workup of Cushing's syndrome (CS) requires various tests which only capture short-term cortisol exposure, whereas patients with endogenous CS generally have elevated cortisol levels over longer periods of time. Scalp hair assessment has emerged as a convenient test in capturing glucocorticoid concentrations over long periods of time. The aim of this multicenter, multinational, prospective, case-control study was to evaluate the diagnostic efficacy of scalp hair glucocorticoids in screening of endogenous CS. ***Methods:*** We assessed the diagnostic performances of hair cortisol (HairF), hair cortisone (HairE), and the sum of both (sumHairF+E), as measured by a state-of-the-art LC-MS/MS technique, in untreated patients with confirmed endogenous CS (n = 89) as well as in community controls (n = 295) from the population-based Lifelines cohort study. ***Results:*** Both glucocorticoids were significantly elevated in CS patients when compared to controls. A high diagnostic efficacy was found for HairF (area under the curve 0.87 [95% CI: 0.83–0.92]), HairE (0.93 [0.89–0.96]), and sumHairF+E (0.92 [0.88–0.96]) (all $p < 0.001$). The participants were accurately classified at the optimal cutoff threshold in 86% of the cases (81% sensitivity, 88% specificity, and 94% negative predictive value [NPV]) by HairF, in 90% of the cases (87% sensitivity, 90% specificity, and 96% NPV) by HairE, and in 87% of the cases (86% sensitivity, 88% specificity, and 95% NPV) by the sumHairF+E. HairE was shown to be the most accurate in differentiating CS patients

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from controls. ***Conclusion:*** Scalp hair glucocorticoids, especially hair cortisone, can be seen as a promising biomarker in screening for CS. Its convenience in collection and workup additionally makes it feasible for first-line screening.

Introduction

Cushing's syndrome (CS) results from excessive exposure to glucocorticoid hormones and is associated with significant morbidity and mortality [1]. After exclusion of exogenous CS caused by glucocorticoid-containing drugs, a variety of endogenous diseases can give rise to increased secretion of cortisol. Approximately 70% of the cases of endogenous CS are caused by a pituitary adenoma producing excessive ACTH, stimulating the adrenal to produce cortisol (i.e., Cushing's disease). The remainder of endogenous CS cases mostly consist of adrenal causes and ectopic ACTH production [1].

Endogenous CS is rare but often presents with common and therefore nonspecific signs and symptoms such as weight gain, fatigue, metabolic syndrome features, and depression [2]. Features more specific for CS include easy bruising, facial plethora, and proximal myopathy, but these do not occur in all patients [3]. This clinical dilemma can cause a significant delay in diagnosis, which is often made when the condition has been existing for an extended period of time and patients display multiple signs and symptoms of CS. Current guidelines recommend three different first-line screening tests: 24-h urinary free cortisol (UFC), late-night salivary cortisol (LNSC), and the 1-mg dexamethasone suppression test [4]. All three tests rely on patient compliance for the collection of samples or drug intake, and their limitations often necessitate repeated testing. Furthermore, they may be influenced by several factors such as kidney function (for UFC), gingival microtrauma (for LNSC), and drug use (for the dexamethasone suppression test).

Recently, we reported on the largest study thus far using measurements of scalp hair cortisol in patients with CS [5]. Scalp hair offers information about integrated cortisol exposure over months of time [6]. This may be particularly valuable in CS, where cortisol production may often vary across days. In our study, hair cortisol provided a 93% sensitivity and 91% specificity for CS, comparing well to first-line tests [5]. Furthermore, hair analysis can be used to create retrospective timelines of cortisol exposure, which can be helpful in cases of cyclic CS [7, 8].

All studies measuring hair cortisol in CS thus far relied on immunoassays to quantify cortisol. A recent advance in the development of hair steroid analysis is hair steroid profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Recently, we have validated a method which measures hair values of cortisol, cortisone, testosterone, androstenedione, dehydroepiandrosterone sulfate, and 17α-hydroxyprogesterone [9]. In contrast to immunoassays, LC-MS/MS is less prone to interference, offers higher sensitivity, and can be used to measure multiple steroids simultaneously. The aim of this study was to assess the diagnostic efficacy of hair cortisol (HairF) and cortisone (HairE) measured by LC-MS/MS in two independently collected cohorts of patients with endogenous CS.

Subjects and Methods

Study Participants

Our study population consisted of 295 controls from the general Dutch population, which had also been included in our previous study [10], and 89 patients with proven endogenous CS. All controls were recruited from Lifelines, which is a multidisciplinary, prospective, population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the north of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics [11]. Patients were recruited from two clinic sites, one in the Netherlands (Erasmus MC, Rotterdam; $n = 19$) and one in Germany (Klinikum der Ludwig-Maximilians-Universität München, Munich; $n = 70$). Diagnostic workup was performed according to the guideline [4] and the diagnosis of CS, de novo or recurrent, was biochemically established by experienced endocrinologists and proven by surgery and/or additional investigations (e.g., bilateral inferior petrosal sinus sampling).

Scalp Hair Measurements

In all participants, a scalp hair sample of approximately 100–150 hairs was collected from the posterior vertex. The hair was cut as close to the scalp as possible and after sample collection stored in an envelope in the dark at room temperature. The protocol for hair processing and analysis was adapted from the previous method described in detail elsewhere [9]. In short, approximately 20 mg of the proximal 3 cm (or the entire length of the hair sample, if the hair was shorter than 3 cm) was weighed and cut into 1-cm-long pieces. The hair was washed in 2 mL of LCMS-grade isopropanol for 2 min and allowed to fully dry. Steroids were extracted overnight in 1.4 mL of LCMS-grade methanol, and 100 µL of cortisol-d3 and cortisone-d8 as internal standards for 18 h at 25 °C while the samples were being gently shaken. After extraction, hair samples were centrifuged at 4,369 *g* (4,500 rpm) for 5 min, and 900 µL of the extract was transferred to a clean tube. We then added 750 µL of methanol to the hair samples, which were spun down again,

Table 1. Descriptive characteristics and hair glucocorticoids of the controls and Cushing's syndrome patients

	Controls (n = 295)	Cushing's syndrome patients		
		cohort 1 (n = 19)	cohort 2 (n = 70)	combined (n = 89)
Female	220 (74.6)	16 (84.2)	50 (71.4)	66 (74.2)
Age, years	42.3±11.5	44.2±16.7	51.8±15.4	50.2±15.9
Hair glucocorticoids, pg/mg				
Hair cortisol (HairF)	2.7 (2.5–2.9)	17.3 (9.5–31.3)	11.7 (8.5–16.2)	12.7 (9.6–16.9)
Hair cortisone (HairE)	8.2 (7.8–8.7)	37.9 (21.7–66.3)	40.9 (30.8–54.4)	40.2 (31.4–51.5)
Sum hair glucocorticoids (sumHairF+E)	11.2 (10.6–12.0)	63.7 (39.4–102.9)	49.7 (38.1–65.0)	52.6 (41.8–66.2)

Data are shown as n (%), mean ± SD, or geometric mean (95% CI).

after which another 900 μL of extract was transferred to the tubes with the extract. The extracts were evaporated under a continuous nitrogen stream at 37 °C, reconstituted in 1 mL of purified water and 20 μL of methanol, and purified using solid-phase extraction.

Cortisol and cortisone concentrations were subsequently quantified by LC-MS/MS using a Xevo TQ-S system (Wates, Milford, MA, USA). HairF and HairE were successfully determined in 91 and 97% of the study participants. Data on both hair glucocorticoids were available for 89% of the study population. The interassay coefficient of variation for cortisol and cortisone was 14.8 and 15.3%, respectively. The intra-assay coefficient of variation for cortisol and cortisone was <11 and <8%, respectively. The lower limit of quantification of cortisol and cortisone was <1.3 and <9.3 pg/mg, respectively. For research purposes, HairF and HairE measurements below the lower limit of quantification were included in the analyses as quantitative measures, since no recognized substitution method exists.

Statistical Analysis

We used SPSS version 24 (IBM Corp., Armonk, NY, USA) and RStudio version 1.0.136 (RStudio, Inc., Boston, MA, USA) with the pROC package [12] for the statistical analyses. The hair glucocorticoid values were logarithmically transformed to achieve a normal distribution and are reported as geometric means and 95% CI. The baseline characteristics were analyzed using ANCOVA if continuous, and using χ^2 tests if categorical. Associations between HairF and HairE were assessed by Pearson's correlation. The diagnostic efficacy of HairF, HairE, and the sum of HairF and HairE (sumHairF+E) for CS screening was assessed using receiver operating characteristic (ROC) curves.

Optimal cutoffs, defined as the curve points closest to the top-left corner, were initially determined for cohorts 1 and 2 separately. For the main analyses, both cohorts were combined and optimal cutoff values were determined for the complete population. DeLong's test was used to compare ROC curves between the two cohorts. Paired analyses were additionally performed to assess the discriminating ability of the different outcomes relative to each other. Moreover, we computed the diagnostic accuracy (i.e., the percentage of correctly classified subjects) and other diagnostic performance parameters (i.e., sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], positive likelihood ratio [LR^+], and negative likelihood ratio [LR^-]). Given the intraindividual and interassay coefficients of variation, we additionally calculated diagnostic performance parameters at 15 and 30% higher and lower levels than the optimal cutoffs. Furthermore, we performed sensitivity analyses in order to account for potential effects of exogenous glucocorticoids on hair glucocorticoid concentrations [10]. We repeated the main ROC analyses with only nonusers in the control cohort. This resulted in exclusion of a total of 38 controls who had used any type of exogenous glucocorticoids in the previous 3 months. Among these participants, hair analyses were successful in 36/38 for HairF and sumHairF+E, and in 37/38 for HairE. All outcomes were considered statistically significant in case of a p value <0.05.

Results

Descriptive Characteristics and Hair Glucocorticoid Concentrations

The subjects' characteristics and concentrations of hair glucocorticoids are shown in Table 1 and Figure 1. On average, the controls were younger (42.3 years) than the patients (50.2 years). The majority of the participants were women in both the control group (74.6%) and the CS group (74.2%). Hair glucocorticoids stratified by sex are shown in online supplementary Table S1 (see www.karger.com/doi/10.1159/000498886 for all online suppl. material). In general, men had higher levels on all measures; however, significant sex differences in the three indices were only present in the controls. Both male and female CS patients had higher values than the controls of same sex (all $p < 0.001$). Overall, there was a strong linear association between HairF and HairE ($r = 0.821$, $p < 0.001$). The geometric mean HairF was higher in the CS patients of cohort 1 (17.3 pg/mg [95% CI: 9.5–31.3]) and cohort 2 (11.7 pg/mg [95% CI: 8.5–16.2]) than in the controls (2.7 pg/mg [95% CI: 2.5–2.9]) (both $p < 0.001$). HairE was also significantly higher in the patients (cohort 1: 37.9 pg/mg [95% CI: 21.7–66.3]; cohort 2: 40.9 pg/mg

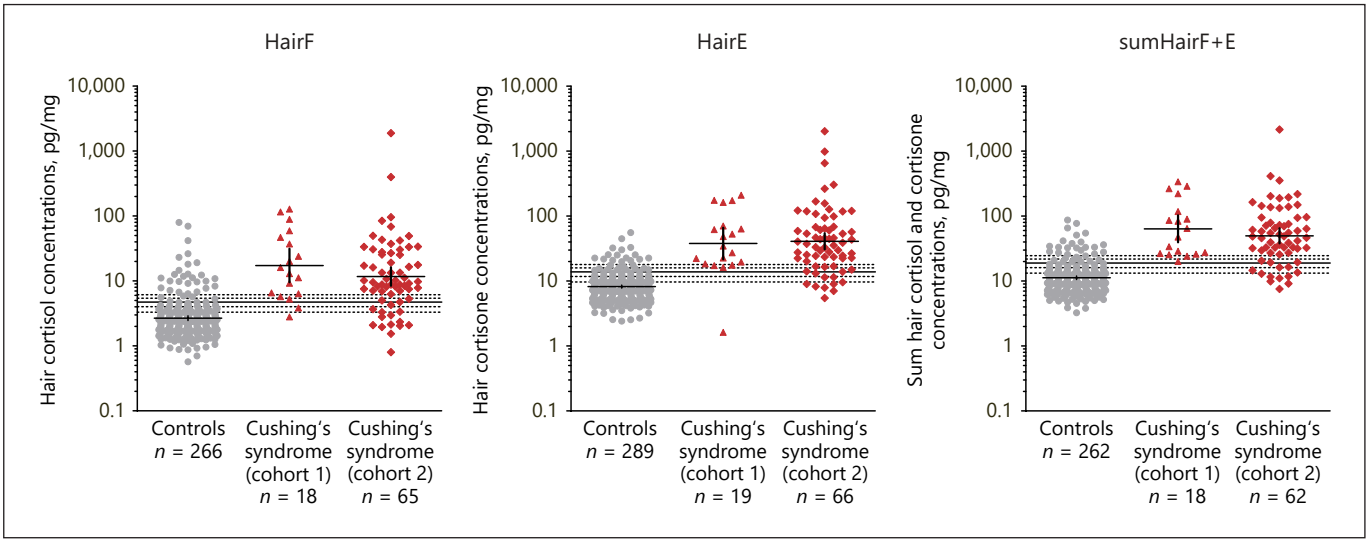


Fig. 1. Distribution of hair glucocorticoid concentrations in controls and Cushing's syndrome patients. Hair cortisol (HairF), hair cortisone (HairE), and the sum of both (sumHairF+E) are shown for community controls, as well as for Cushing's syndrome patients from two independent cohorts. The data for each group are summarized as the geometric mean with corresponding 95% CI. The solid black lines correspond to the optimal cutoff values, and the dashed lines above and below indicate levels corresponding to 15 and 30% above and below the optimal cutoff values, respectively.

[95% CI: 30.8–54.4]) than in the controls (8.2 pg/mg [95% CI: 7.8–8.7]) (both $p < 0.001$). The geometric mean of the sum of both hair glucocorticoids was also higher in the CS patients than in the controls. There were no statistically significant differences in hair glucocorticoids between the two patient cohorts.

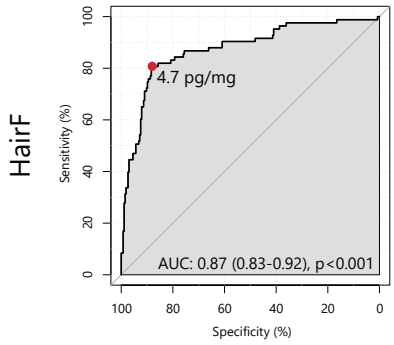
Diagnostic Efficacy of Hair Glucocorticoids for Screening of CS

ROC curves with corresponding diagnostic performance parameters for HairF, HairE, and sumHairF+E are depicted in Figure 2. Analyses stratified by sex are shown in online supplementary Figure S1. All three indices showed a strongly significant differentiating efficacy among CS patients from both cohorts separately and combined ($p < 0.001$ for all areas under the curve [AUCs]).

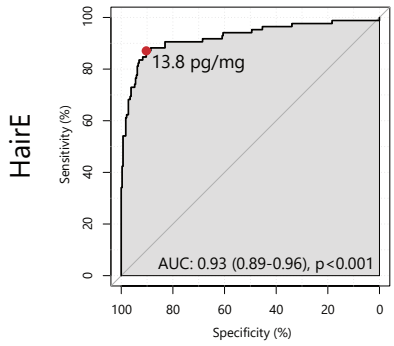
For HairF, an optimal cutoff of 4.7 pg/mg (AUC 0.87 [95% CI: 0.83–0.92]) was observed, with an accuracy of 86%, a sensitivity of 81%, and a specificity of 88%. A positive test result confirmed CS with 68% probability, whereas the NPV was 94%. In regard to HairE, the ROC analysis yielded an optimal cutoff of 13.8 pg/mg (AUC 0.93 [0.89–0.96]). This allowed the correct identification of 74/85 CS patients and 261/289 controls, corresponding to 90% accuracy, 87% sensitivity, and 90% specificity. The PPV and NPV with HairE was 73 and 96%, respectively. The sum of both hair glucocorticoids also showed a high diagnostic efficacy with an AUC of 0.92 (95% CI: 0.88–0.96). The optimal sumHairF+E cutoff was 18.9 pg/mg, with a corresponding sensitivity of 86% and a specificity of 88%. At this cutoff, 69/80 CS patients and 230/262 controls were identified correctly, yielding an accuracy of 87% with a PPV of 68% and an NPV of 95%.

In the context of sensitivity analyses to take potential influencing effects of glucocorticoid-containing drugs into account, we found nearly identical AUCs when only nonusers were considered as controls ($p < 0.001$ for all three indices; data not shown). Moreover, the optimal cutoff levels with corresponding sensitivity and specificity were also roughly the same for HairF (4.7 pg/mg; 81% sensitivity, 87% specificity), HairE (13.8 pg/mg; 87% sensitivity, 89% specificity), and sumHairF+E (16.2 pg/mg; 89% sensitivity, 85% specificity). Diagnostic accuracy at these levels was 86% for HairF, 89% for HairE, and 86% for sumHairF+E.

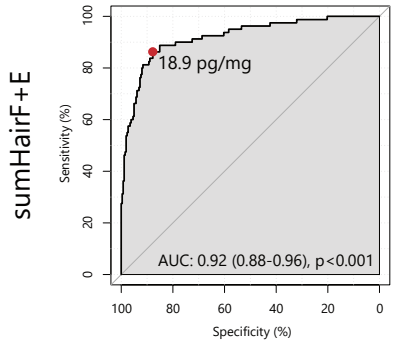
The optimal cutoff for all outcomes was lower in cohort 2 than in cohort 1; however, only the sum of hair glucocorticoids was statistically significantly different in diagnostic efficacy between the two cohorts (Fig. 3). Paired ROC analysis of the hair glucocorticoids showed that HairE and sumHairF+E were more accurate than HairF in the screening of CS in the complete study population (both $p < 0.010$; Fig. 4), whereas HairE was marginally more accurate than the sum value ($p = 0.041$).



	Value (pg/mg)	Accuracy (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR positive	LR negative
OC-30%	3.3	78	86.7	75.6	52.6	94.8	3.6	0.2
OC-15%	4.0	83	81.9	83.5	60.7	93.7	5.0	0.2
OC	4.7	86	80.7	88.0	67.7	93.6	6.7	0.2
OC+15%	5.4	86	75.9	88.7	67.7	92.2	6.7	0.3
OC+30%	6.1	86	74.7	89.8	69.7	91.9	7.4	0.3



	Value (pg/mg)	Accuracy (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR positive	LR negative
OC-30%	9.7	74	90.6	69.2	46.4	96.2	2.9	0.1
OC-15%	11.8	86	88.2	85.8	64.7	96.1	6.2	0.1
OC	13.8	90	87.1	90.3	72.5	96.0	9.0	0.1
OC+15%	15.9	91	83.5	93.1	78.0	95.1	12.1	0.2
OC+30%	18.0	90	76.5	94.5	80.2	93.2	13.8	0.3



	Value (pg/mg)	Accuracy (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR positive	LR negative
OC-30%	13.3	75	91.3	70.6	48.7	96.4	3.1	0.1
OC-15%	16.1	85	88.8	84.4	63.4	96.1	5.7	0.1
OC	18.9	87	86.3	87.8	68.3	95.4	7.1	0.2
OC+15%	21.8	88	81.3	90.5	72.2	94.0	8.5	0.2
OC+30%	24.6	89	80.0	92.0	75.3	93.8	10.0	0.2

Fig. 2. Receiver operating characteristic curve analyses of the diagnostic performance of hair glucocorticoids for Cushing's syndrome. The red dots refer to the OC value for screening of Cushing's syndrome. The table summarizes the different diagnostic performance parameters at the OC level and other specified levels. AUC, area under the curve; HairE, hair cortisone concentrations; HairF, hair cortisol concentrations; sumHairF+E, sum of HairF and HairE; OC, optimal cutoff threshold; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

Discussion

In this multicenter study, we evaluated, for the first time, the diagnostic efficacy of scalp hair cortisol and cortisone concentrations as measured by LC-MS/MS for the screening of CS in two independent patient cohorts. We showed that both glucocorticoids were significantly elevated in patients when compared to community controls, while there were no differences between the patient cohorts. With respect to diagnostic performance, we found a high differentiating capacity of HairF (accuracy 86%, sensitivity 81%, and specificity 88%), HairE (accuracy 90%, sensitivity 87%, and specificity 90%), and the sum of both (accuracy 87%, sensitivity 86%, and specificity 88%). Excluding users of exogenous glucocorticoids in the control cohort revealed no significant effects on these findings. Paired analyses

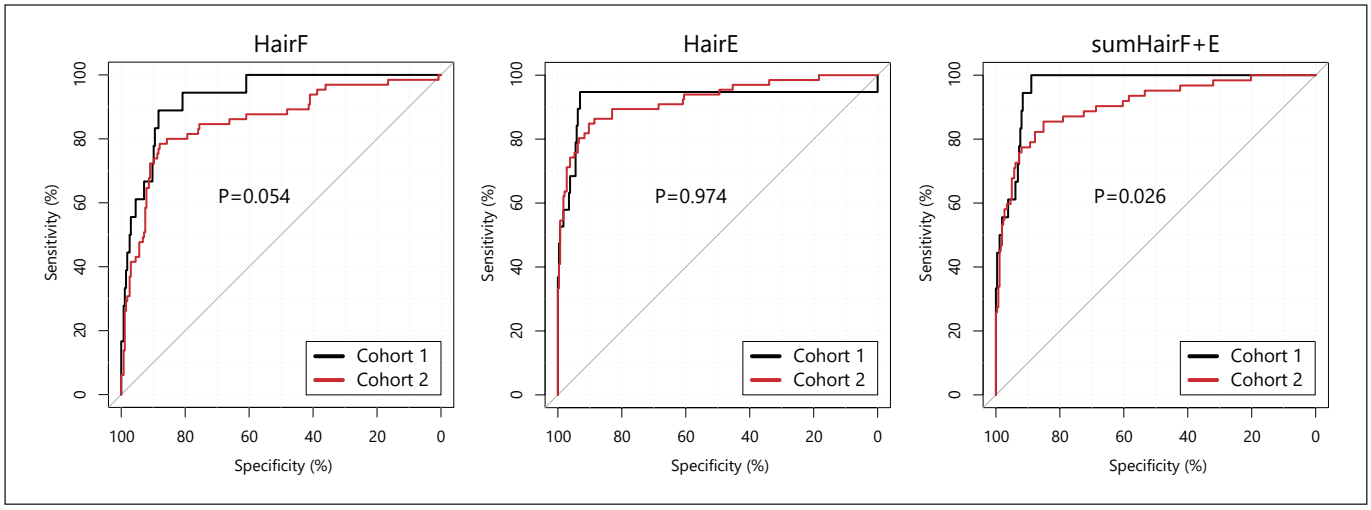


Fig. 3. Comparison of receiver operating characteristic curves for screening of Cushing's syndrome by hair glucocorticoids between two independent patient cohorts. HairE, hair cortisone concentrations; HairF, hair cortisol concentrations; sumHairF+E, sum of HairF and HairE.

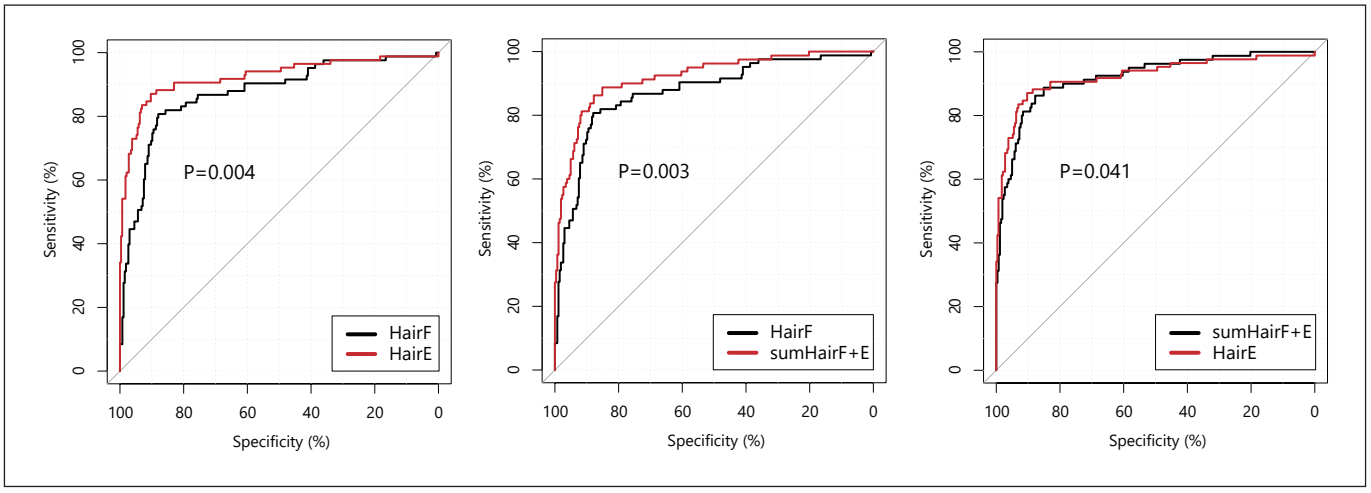


Fig. 4. Paired analyses for differences in diagnostic efficacy between hair glucocorticoids for screening of Cushing's syndrome. Hair cortisone (HairE) was more accurate than hair cortisol (HairF) and the sum of both glucocorticoids (sumHairF+E) in differentiating patients with Cushing's syndrome from controls. sumHairF+E was also statistically significantly better than HairF with respect to diagnostic efficacy.

showed that HairE was more accurate than HairF or the sum of both in distinguishing patients from controls.

Assessment of cortisol concentrations in scalp hair has previously been performed by us and others to compare levels between CS patients and controls [5, 7, 8, 13, 14]. Published studies consistently showed clearly elevated levels in patients in the proximal 1- and 3-cm hair segments. Recently, we have also investigated the diagnostic efficacy of HairF in distinguishing CS patients from healthy controls, as well as patients suspected of CS but in whom the diagnosis was eventually excluded. High sensitivity and specificity were observed with similar optimal cutoffs for both analyses [5]. However, this and previous studies have only analyzed HairF and have performed analyses by immunoassay, which is, among others, prone to cross-reactivity and is inferior to LC-MS/MS with respect to selectivity and detection. Findings of local production of 11β-hydroxysteroid dehydrogenase (11β-HSD) types 1 and 2 – which are, respectively, responsible for the conversion of cortisone into cortisol and vice versa – in

skin, hair follicles, and other cutaneous appendages [15–17] also complicate the interpretation of prior findings in CS patients. It therefore remains questionable whether the measured hair cortisol concentrations only reflect the actual past exposure to cortisol or whether these are altered due to local conversion by 11β-HSDs.

Here, we showed for the first time that HairF as well as HairE is elevated in CS patients and that both glucocorticoids possess a high diagnostic efficacy. Moreover, we showed a relatively better diagnostic performance of HairE in distinguishing patients from controls when compared to HairF. Another test that might also be prone to local conversion effects is the first-line screening test with salivary cortisol – this because of the 11β-HSD2 activity in parotid tissue [17]. A previous study by Perogamvros et al. [18] focused on both salivary glucocorticoids in non-cushingoid patients and found, similarly to the current work, higher concentrations of cortisone than of cortisol, whereas the opposite was true for the free fractions in serum. Interestingly, sampling after adrenal stimulation with ACTH injection showed salivary cortisone to reflect free serum cortisol more accurately than salivary cortisol. An evaluation of salivary cortisol and cortisone in another study with CS patients indeed revealed a high diagnostic accuracy of both measures [19]. Additionally, a recent study by Kapoor et al. [20] with radiolabeled cortisol experiments on primates confirmed that circulating cortisol is taken up in hair and can be measured. Importantly, the authors also showed that a substantial proportion of the administered cortisol was incorporated as cortisone. More research, however, is needed to understand the dynamics between cortisol and cortisone at the local level and to investigate the additional value of cortisone measurements.

The diagnostic efficacy of screening tests depends on the chosen cutoff value for differentiating patients from subjects without the disease. This makes it challenging to place our results in the context of the recommended tests. Nevertheless, Elamin et al. [21] have systematically summarized and pooled the results of the traditional tests in the diagnostic workup of CS. Based on this, the diagnostic efficacy of hair glucocorticoids, especially of HairE, seems to be quite similar to that of midnight salivary cortisol (pooled LR^+ 8.8 and LR^- 0.1) and UFC (pooled LR^+ 10.6 and LR^- 0.2), even though most of the included studies had a small population with a fairly high prevalence of CS [21]. Since the diagnosis of CS could not rely on a single screening test, further research should especially address the diagnostic effectiveness of hair glucocorticoids in combination with other recommended tests. Besides, as mentioned in the guideline and also observed here, there is a substantial proportion of false positives with the screening tests, due among other things to the high prevalence of (mild) hypercortisolistic cushingoid-like conditions (e.g., psychiatric disorders, diabetes mellitus, and obesity) and the rare occurrence of CS [4]. Therefore, the recommendation to restrict testing to subjects with a high a priori probability of having CS could reasonably be extended to hair glucocorticoid assessment.

The current screening tests are subject to several difficulties and limitations which are less severe or completely absent with scalp hair measurements. From the patient's perspective, hair sampling is noninvasive and does not require following specific instructions (e.g., collection of urine output for at least 24 consecutive hours for UFC) or impose restrictions (e.g., fasting or no teeth brushing before saliva collection for LNSC) as with the recommended tests; also, hair samples can be collected, stored, and posted by mail with ease, which is especially useful for patients who have to cover long distances to a clinic site. For care professionals, it is convenient that hair measurements are not dependent on the time of day or patient compliance and are not influenced by acute stressors. The unique feature of these measurements of covering long-term glucocorticoid exposure makes them additionally useful in the screening for cyclical CS. The current guideline recommends UFC or salivary cortisol measurements in case of suspicion of cyclical CS [4]; however, these tests can yield normal results when patients are screened after the periodical increase in cortisol levels. We previously demonstrated the usefulness of hair measurements in such situations in multiple patients who had normal screening test results at the time of evaluation but had retrospectively elevated cortisol concentrations in hair segments corresponding to the period of cushingoid signs and symptoms [7].

The large number of patients and controls and the multicenter evaluation are among the major strengths of the current work. Moreover, all hair glucocorticoid concentrations were determined with high sensitivity and specificity using a state-of-the-art LC-MS/MS technique. This study is, however, limited in the way that controls from the community were not screened for CS. Nevertheless, given the rarity of this disorder, with less than 5 cases per million individuals [22], it is very unlikely that controls were misclassified. Moreover, the results were not adjusted for potential confounders such as UV exposure [23], hair washing, or diabetes mellitus [24]. It is, however, questionable whether these factors would have substantially influenced the outcomes, because of the large (5–6 fold) differences between controls and CS patients in hair glucocorticoid levels.

In conclusion, scalp hair assessment for hair glucocorticoids, in particular for cortisone concentrations, shows a high diagnostic efficacy in differentiating CS patients from controls. Because of its simplicity and noninvasive sampling, as well as its diagnostic performance, it may be seen as a promising biomarker and a potential addition to the armamentarium of CS screening tests. To allow the uniform use of fixed cutoff values, we recommend further efforts to standardize or harmonize results between international centers.

Statement of Ethics

Written informed consent was obtained from all participants. This study was approved by the medical ethics committees of both institutions.

Disclosure Statement

The authors have no conflicts of interest to declare.

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